Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

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Table of Contents

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1. Introduction

This is an *in vivo* method which employs challenge of swine to determine the potency of killed pseudorabies virus (PRV) vaccines or the immunogenicity of PRV Master Seed viruses as specified in the Code of Federal Regulations, Title 9 (9 CFR).

2. Materials

2.1 Equipment/instrumentation

2.1.1 Digital thermometer[1]

2.1.2 Centrifuge[2] with rotor[3]

2.1.3 Water bath[4]

2.2 Reagents/supplies

2.2.1 PRV-susceptible swine at the minimum age recommended for vaccination and shown to be negative for PRV serum neutralizing (SN) antibodies by the current version of MVSAM0117. Swine shall be considered susceptible if there is no neutralization at a final serum dilution of 1:2. Other tests of equal sensitivity acceptable to the Animal and Plant Health Inspection Service (APHIS) may be used.

2.2.1.1 Numbers of animals required for testing:

1. 9 CFR 113.213: 5 vaccinated swine (VS) + 5 controls (CONT)

2. 9 CFR 113.318(b)(4): 5 VS + 5 CONT

3. 9 CFR 113.318(b)(1)-(3): 20 VS + 5 CONT

2.2.2 PRV Challenge, Becker strain[5]

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[1] Model M216, GLA Agricultural Electronics, 4120 Horizon Ln., San Luis Obispo, CA 93401 or equivalent
[2] Model J6B, Beckman Instruments, Inc., 2500 Harbor Blvd., Box 3100, Fullerton, CA 92834-3100 or equivalent
[3] Model JS-4.0 Beckman Instruments, Inc. or equivalent
[4] Cat. No. 15-461-10, Fisher Scientific Corp., 2000 Park Ln., Pittsburg, PA 15275 or equivalent
[5] Available upon request from the Center for Veterinary Biologics-Laboratory, P.O. Box 844, Ames, IA 50010 or approved by APHIS

2.2.3 Minimum Essential Medium (MEM)

2.2.3.1 9.61 g MEM[6]

2.2.3.2 2.2 g sodium bicarbonate ($NaCHO_3$)[7]

2.2.3.3 Q.S. to 1000 ml with deionized water, adjust pH to 6.8-6.9 with 2N hydrochloric acid (HCl)[8]

2.2.3.4 Sterilize through a 0.22-µm filter.[9]

2.2.3.5 Store at 4° ± 2°C.

2.2.4 Serum separation tubes[10] with 20-ga x 0.5-in Vacutainer® needles[11]

2.2.5 Polystyrene tubes, 12 x 75 mm[12]

2.2.6 Syringes, 3 and 10 ml[13] and needles[14]

3. Preparation for the test

3.1 Personnel qualifications/training

Personnel must have training and experience in evaluating swine for clinical signs of disease due to PRV infection. **A current Animal Use Application must be available prior to ordering the swine for the test.** All constraints in the application must be followed.

3.2 Preparation of equipment/instrumentation

3.2.1 Set the water bath at 36° ± 2°C.

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[6] MEM with Earle's salts without sodium bicarbonate, Cat. No. 410-1500EF, Life Technologies, Inc., 8400 Helgerman Ct., Gaithersburg, MD 20884 or equivalent
[7] Cat. No. S 5761, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 or equivalent
[8] Cat. No. 9535-01, J.T. Baker, Inc., 222 Red School Ln., Phillipsburg, NJ 08865 or equivalent
[9] Cat. No. 12122, Gelman Sciences, 600 S. Wagner Rd., Ann Arbor, MI 48106 or equivalent
[10] Vacutainer® 6512, Becton Dickinson Labware, 1 Becton Dr., Franklin Lakes, NJ 07417-1885 or equivalent
[11] Vacutainer® 7290, Becton Dickinson Labware or equivalent
[12] Falcon 2058, Becton Dickinson Labware or equivalent
[13] Luer-Lok®, Cat. No. 309585 & 309604 respectively, Becton Dickinson Labware or equivalent
[14] 20 ga, 1½ in, Cat. No. 250107, Becton Dickinson Labware or equivalent

3.3 Preparation of reagents/control procedures

3.3.1 Rapidly thaw the PRV Challenge in a 36° ± 2°C water bath; dilute in MEM as recommended on the CVB-L Reference and Reagent Data Sheet.

3.4 Preparation of the sample

3.4.1 No preparation of Test Serial/Master Seed is required.

3.4.2 Preparation of blood samples.

1. Allow blood samples to clot in the serum separation tubes at room temperature (23° ± 2°C) for 20 ± 5 min.

2. Separate serum from the clot by centrifuging the tubes at 1000 X *g* for 20 ± 5 min (22,000 rpm, Model J6B centrifuge with a JS-4.0 rotor).

3. Pour off the serum into labeled 12 x 75-mm polystyrene tubes. Maintain each animal's serum separately.

4. Store serum samples at -20° ± 5°C until tested for SN antibodies according to the current version of MVSAM0117.

4. Performance of the test

4.1 On the day of the first vaccination, bleed, using the Vacutainer® system, from the anterior vena cava of all VS and CONT swine for SN antibody susceptibility determination. Using a 10-ml syringe and needle, administer 1 dose of Test Serial/Master Seed as recommended on the label to all VS. Replace the needle between swine. Follow label recommendations for interval between vaccinations if 2 doses are to be administered to the swine; repeat the vaccination if required. **NOTE: CONT swine are not vaccinated.**

Supplemental Assay Method for Pseudorabies Virus Challenge Test in Swine

4.2 At 14-28 days postvaccination (DPV) after the last vaccination, draw blood samples, using the Vacutainer® system, from the anterior vena cava of all VS and CONT swine.

4.3 For a Test Serial, if at least 4 of the 5 VS have not developed titers of at least 1:8 and the remaining VS have not developed a titer of 1:4, the VS and CONT swine may be tested by challenging as stated below. For an MSV, all swine are challenged.

4.4 Using a 3-ml syringe without a needle, administer intranasally, 2 ml of the diluted PRV Challenge per swine (1 ml/nostril), during inhalation to both VS and CONT swine. Swine should be held vertically with head up and not sedated or anesthetized for the challenge.

4.5 For -1 and 0 days postchallenge (DPC) exposure, determine and record rectal temperatures.

4.6 Observe swine daily for 1 to 14 DPC in the morning before and during feeding. Note and record all clinical observations. After clinical observations are recorded, determine rectal temperatures and record for 1 to 7 DPC.

4.7 Clinical observations:

4.7.1 For **9 CFR 113.318(b)(3)(i)** CONT swine, severe central nervous system (CNS) signs include, but are not limited to: falling, difficulty rising, inability to rise, head tilt, head pressing, paralysis, tremors, spasms, convulsions, paddling, opisthotonos, circling, and coma.

4.7.2 For **9 CFR 113.213(c)(2)(vi)** CONT swine, CNS signs include all of the signs listed previously: plus pruritus, teeth grinding, empty chewing, persistent or unusual vocalization, disorientation, ataxia, stumbling, loss of postural control, and proprioceptive placing deficits.

4.7.3 Clinical signs of PRV infection in VS include, but are not limited to: all of the CNS signs listed previously (**sections 4.7.1-4.7.2**), plus anorexia on 2 or more consecutive DPC, fever of ≥ 106°F (≥ 41.1°C) on any 2 or more DPC, stunting, weakness, vomiting, diarrhea, constipation on any 2 or more DPC, blindness or other ocular disease, persistent sneezing, persistent or deep coughing, labored breathing, and pneumonia.

4.7.4 For the purposes of this testing, the following clinical signs of PRV infection will not be included in the evaluations, due to their ambiguity: transient inappetence, depression, shivering, occasional sneezing, occasional upper respiratory cough, reduction in rate of weight gain, and fever which is transient or < 106°F (< 41.1°C).

5. Interpretation of the test results

5.1 For swine challenge-exposure no point system or weighted scoring system is allowed.

5.2 Validity requirements:

5.2.1 9 CFR 113.318 (b)(3)(i): If at least 4 of the 5 CONT swine do not develop severe CNS signs or die, the test is inconclusive and may be repeated. For each swine, this development will be considered the observations on 1 or more days as described in **section 4.7.1**.

5.2.2 9 CFR 113.213 (c)(2)(vi): If at least 4 of the 5 CONT swine do not develop CNS signs or die, the test is inconclusive and may be repeated. For each swine, this development will be considered the observations on 1 or more days as described in **section 4.7.2**.

5.3 Test Serial evaluation criteria:

5.3.1 9 CFR 113.213: If 2 or more of the VS develop clinical signs of PRV infection or die, the Test Serial is unsatisfactory.

5.3.2 9 CFR 113.318(b): If 2 or more of the VS develop clinical signs of PRV infection or die, the Master Seed is unsatisfactory.

6. Report of test results

6.1 Record all test results on a test record.

7. References

7.1 Code of Federal Regulations, Title 9, Parts 113.213 and 113.318, U.S. Government Printing Office, Washington, DC, 1998.

7.2 Vannier P. Experimental infection of fattening pigs with pseudorabies (Aujeszky's disease) virus: efficacy of attenuated live and inactivated virus vaccines in pigs with or without passive immunity. *Am J Vet Res* 1986; **46:**1478-1502.

8. Summary of Revisions

8.1 This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol.